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2. Fox et al., Lipids (2000), 35 (1) : 23-30.

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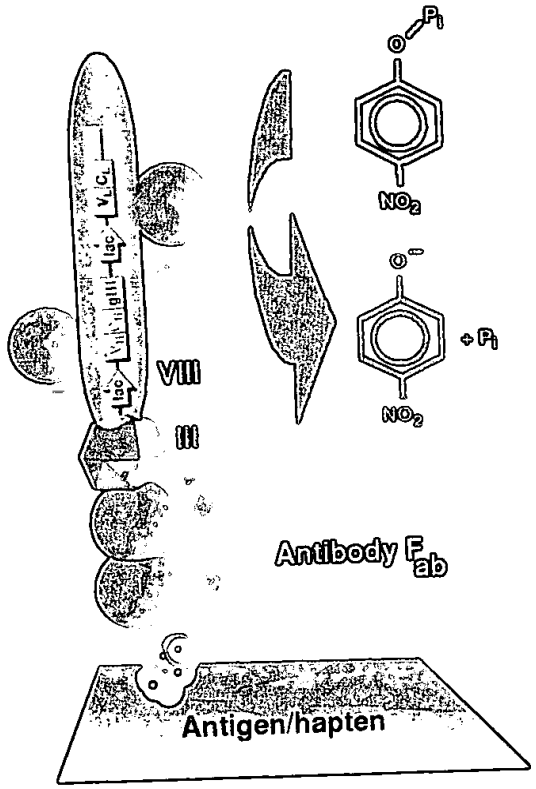
Bioorganic & Medicinal Chemistry Letters



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On the Microbial Biogeneration of (R) γ–Jasmolactone

Claudio Fuganti, Piero Grasselli, Gioia Zucchi

Dipartimento di Chimica del Politecnico, Centro CNR per
la Chimica delle Sostanze Organiche Naturali, 20133 Milano, Italy

Gianna Allegrone, Massimo Barbeni

San Giorgio Flavors, 10147 Torino, Italy

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Abstract The biogeneration of (R) Z-7-decen-4-olide (γ-jasmolactone) (**6**) in *Pichia stipitis* and *ohmeri* from one of the products derived from linolenic acid via photooxidation/reduction is reported

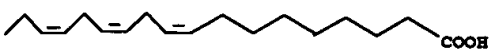
γ-Jasmolactone (Z-7-decen-4-olide) has recently been found as a constituent of and an important contributor to the fruit aroma of white peaches as described by, among others,[1] two of us (G.A. and M.B.).[2] This induced us to explore possible natural procedures leading to this rare compound.[3] In the present note we outline our results, together with the experimental evidence which allowed us to assign the (R) absolute configuration depicted in structural formula (**6**) to the major enantiomer present in white peaches and in the biogenerated material. Simple structural considerations suggest for γ-jasmolactone (**6**) and for its δ analog (**5**) that they are biogenetically derived from linolenic acid. Therefore, it seemed reasonable that these C-10 lactones could be accessible by microbial degradation of hydroxylated derivatives of linolenic acid, in analogy with recent findings, which have shown that a whole set of lactones, including the saturated analogs of the above materials, can be obtained by these means from linoleic and oleic acid.[4,5,6] Therefore, linolenic acid (**1**) (85%, purity, major impurities: linoleic and oleic acids) was submitted to photooxidation in the presence of cercosporin,[4] followed by reduction of the intermediate hydroperoxydes, with L-cysteine which afforded the corresponding alcohols. According to reported data[7] the product mixture is expected to contain, along with the 12 and 13-hydroxylated products (**2**) and (**3**), a mixture of isomeric triply unsaturated acids bearing hydroxyl functions at positions 9, 10, 15 and 16, respectively, in approximately an 12:14:23:13:13:25 ratio. Whereas starting from (**3**), we would expect to obtain directly δ-jasmolactone through C-2 degradation via β oxidation, the conversion of (**2**) into γ-jasmolactone requires an additional reaction step, namely saturation of the double bond present in the C-18 precursor at position 13. This could presumably be accomplished at a late stage from the doubly unsaturated material (**4**). Two microorganisms, *P. ohmeri* and *P. stipitis*, have recently been shown to be able to saturate double bonds in the α-position of the side chain of lactones (i.e., E-5-decen-4-olide is converted into decan-4-olide).[6] Therefore, the biodegradation of the two hydroxyacids (**2**) and (**3**) from above was studied using

these organisms and the crude hydroxyacid mixture from above as the substrate. The lactones produced in the two microorganisms after 24 h incubation of the above mixture are reported in the Table.

Table Lactones isolated at 24 h incubation in *P. ohmeri* and *P. stipitis* of the hydroxy acids obtained from 85% linolenic acid

Entry	lactone	compound	*Pichia ohmeri*	*Pichia stipitis*
1	hexan-4-olide		8.25	3.2
2	5-octen-4-olide	**10**	-	11.9
3	octan-4-olide		-	4
4	nonan-4-olide		5.9	-
5	Z-7-decen-4-olide	**6**	1.1	0.3
6	E-5-decen-4-olide		7.6	8.6
7	decan-4-olide		1.5	-
8	E-5, Z-7-decadien-4-olide	**4**	20.5	25.09
9	Z-7-decen-5-olide	**5**	12.3	12.5
10	Z-6-dodecen-4-olide		11.8	11
11	Z-6, Z-9-dodecadien-4-olide		25.6	16.7
12	dodecan-4-olide		4.6	4
13	5-tetradecen-4-olide	**11**	-	2.6

Considering the nature of the precursor mixture used, the biogenerated products (Table) can be divided in three sets.[8] The products of entries 1, 2, 3, 5, 8, 9 and 11, of entries 4,6,7 and 10, and, finally, of entries 12 and 13, are derived from linolenic, linoleic and oleic acids, respectively. It thus appears, that after 24 h of incubation, using either of the two microorganisms, there is a small but significant production of the Z-7-decen-4-olide (**6**), along with a major amount of E-5-Z-7-decadien-4-olide (**4**). GLC analysis on a chiral capillary column indicated that Z-7-decen-4-olide (**6**) produced in *P. ohmeri* and *P. stipitis*, respectively, was a 85:15 and 83:17 mixture of two enantiomers. The (R) configuration was assigned to the major component which is depicted in (**6**). The assignement was based on the following evidence. Baker's yeast reduction of 4-oxo-Z-7-decenoic acid (**7**) gave enantiomerically pure Z-7-decen-4-olide (**6**), identical to the major product obtained both from the microbial transformations and from peaches.[1,2] Catalytic hydrogenation of the baker's yeast derived material gave rise to the known (R) decan-4-olide[6] thus confirming the assignments of the (R) configuration to all the interrelated products mentioned above. Decan-4-olide is formed through a similar exocyclic double bond saturation mediated by *P. ohmeri* using E-5-decen-4-olide as substrate (entries 6 and 7). The product distribution in the two microbial systems thus observed (Table) requires further comments. For the two systems, four common components (entries 8-11) account for over 60% of the product mixture. The lactones under entries 8, 9 and 11 arise from linolenic acid, whereas Z-6-dodecen-4-olide is formed from linoleic acid. However, of particular interest is the formation of nonan-4-olide when using *P. ohmeri* and of 5-octen-4-olide and 5-tetradecen-4-olide when using *P. stipitis*. The C-9 γ-lactone might be formed by α oxidation of 5-hydroxy decanoic acid, which is the known intermediate on the



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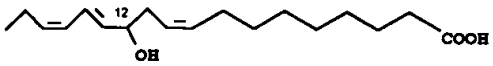

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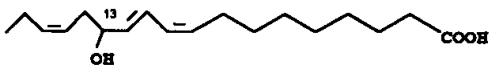

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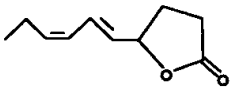

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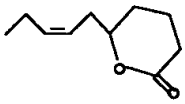

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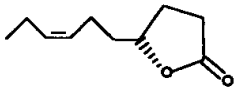

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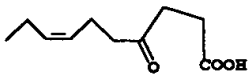

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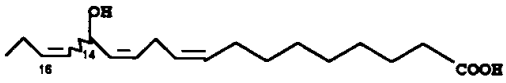

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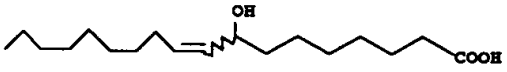

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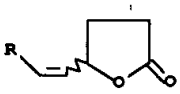

10 R=C_2H_5

11 R=C_8H_{17}

pathway from coriolic acid ((**3**), without unsaturation in position 15) into decan-5-olide.[4] The formation of 5-octen-4-olide (**10**) and 5-tetradecen-4-olide (**11**), when using *P. stipitis* might proceed from the same precursors known to yield hexan-4-olide and dodecan-4-olide, respectively. Thus, the 16 and 9 hydroxy derivatives of linolenic and oleic acids obtained in the photooxidation/reduction are transformed through allylic isomerization to compounds (**8**) and (**9**), respectively, which are subsequently degraded by β oxidation. The Z-isomer of (**11**) is a constituent of *Osmanthus* extracts.[9] Further studies on the stereochemical aspects of the conversions described above are required in order to define the possible participation of enzymic steps in the allylic isomerizations. However, apart from the unresolved mechanistic details, the results obtained here give access to a variety of rare natural lactones. It also constitutes an additional example of the preparative significance of bioconversions in the field of aroma substances.

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10. The derivation of (**11**) from the ca. 1:1 mixture of 9 and 10 hydroxy acids obtained from oleic acid by photooxidation/reduction has been determined through feeding experiments in *P. stipitis* of dideuterated materials[5] which gave dideuterated (**11**).